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Lab Resource: Stem Cell Line

Generation of KCL013 research grade human embryonic stem cell line carrying a mutation in the *HTT* gene

Laureen Jacquet, Heema Hewitson, Victoria Wood, Neli Kadeva, Glenda Cornwell, Stefano Codognotto, Emma Stephenson, Dusko Ilic *

Stem Cell Laboratories, Division of Women's Health, Faculty of Life Sciences and Medicine, King's College London and Assisted Conception Unit, Guys" Hospital, London, United Kingdom

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ABSTRACT

The KCL013 human embryonic stem cell line was derived from an embryo donated for research that carried an autosomal dominant mutation affecting one allele of the *HTT* gene encoding huntingtin (42 trinucleotide repeats; 17 for the normal allele). The ICM was isolated using laser microsurgery and plated on γ-irradiated human foreskin fibroblasts. Both the derivation and cell line propagation were performed in an animal product-free environment. Pluripotent state and differentiation potential were confirmed by in vitro assays.

Resource table

Name of stem cell line	KCL013
Institution	King's College London, London, UK
Derivation team	Neli Kadeva, Victoria Wood, Glenda Cornwell, Stefano Codognotto, Emma Stephenson
Contact person and email	Dusko Ilic, email: dusko.ilic@kcl.ac.uk
Type of resource	Biological reagent: cell line
Sub-type	Human pluripotent stem cell line
Origin	Human embryo
Key marker expression	Pluripotent stem cell markers: NANOG, OCT4, TRA-1-60, TRA-1-81, alkaline phosphatase (AP) activity
Authentication	Identity and purity of line confirmed
Link to related literature (direct URL links and full references)	1) Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy. 14 (1), 122–128.doi: 10.3109/14653249.2011.623692 http://www.ncbi.nlm.nih.gov/pubmed/22029654 2) Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. Nat. Protoc. 7 (7), 1366–1381. doi: 10.1038/nprot.2012.080 http://www.ncbi.nlm.nih.gov/pubmed/22722371

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Name of stem cell line	KCL013
Information in public databases	KCL013 is a National Institutes of Health (NIH) registered hESC line NIH Registration Number: 0214 NIH Approval Number: NIHhESC-13-0214 http://grants.nih.gov/stem_cells/registry/current.htm?id=651
Ethics	The hESC line KCL013 is derived under license from the UK Human Fertilisation and Embryology Authority (research license numbers: R0075 and R0133) and also has local ethical approval (UK National Health Service Research Ethics Committee Reference: 06/Q0702/90). Informed consent was obtained from all subjects and the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the NIH Belmont Report. No financial inducements are offered for donation.

Resource details

Consent signed	Aug 12, 2009
Embryo used	Aug 23, 2009
UK Stem Cell Bank Deposit approval	Sep 23, 2010 Reference: SCSC10-32
Sex	Male 46, XY
Grade	Research
Disease status (Fig. 1)	Mutation affecting one allele of the *HTT* gene encoding huntingtin (~46 CAG repeats; 17 for the normal allele) associated with Huntington's disease (Ilic et al., 2012)

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* Corresponding author.
E-mail address: dusko.ilic@kcl.ac.uk (D. Ilic).

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Karyotype (G banding) (Fig. 2)	No imbalance detected
Karyotype (aCGH)	No imbalance detected
DNA fingerprint	Allele sizes (in bp) of 17 microsatellite markers specific for chromosomes 13, 18 and 21 (Ilic et al., 2012)
Viability testing	Pass
Pluripotent markers (immunostaining) (Fig. 3)	NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity (Ilic et al., 2012)
Three germ layer differentiation in vitro (immunostaining) (Fig. 4)	Endoderm: AFP (α-fetoprotein); ectoderm: TUBB3 (tubulin, β3 class III); mesoderm: ACTA2 (actin, α2, smooth muscle) (Ilic et al., 2012)
Sibling lines available	KCL012

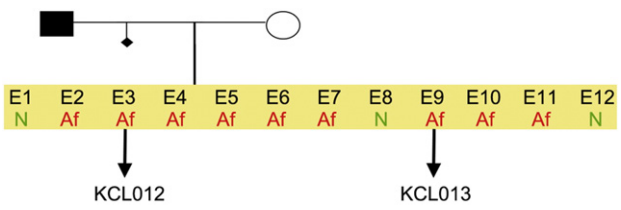

Fig. 1. Genetic pedigree tree. The couple undergoing IVF had 12 embryos in this particular cycle. Three embryos were normal, whereas nine carried the mutation in *HTT* and were donated for research. We derived hESC lines from two of them.

We generated KCL013 clinical grade hESC line following protocols, established previously (Ilic et al., 2012; Stephenson et al., 2012). The expression of the pluripotency markers was tested after freeze/thaw cycle (Fig. 3; Ilic et al., 2012). Differentiation potential into three germ layers was verified in vitro (Fig. 4; Ilic et al., 2012).

Materials and methods

Consenting process

We distribute Patient Information Sheet (PIS) and consent form to the in vitro fertilization (IVF) patients if they opted to donate to research embryos that were stored for 5 or 10 years. They mail signed consent back to us and that might be months after the PIS and consent form were mailed to them. If in the meantime new versions of PIS/consent are implemented, we do not send these to the patients or ask them to re-sign; the whole process is done with the version that was given them initially. The PIS/consent documents (PGD-V.6) were created on Aug. 10, 2007. HFEA Code of Practice that was in effect at the time of document creation: Edition 7 — R.1 (http://www.hfea.gov.uk/2999.html). The donor couple signed the consent on Oct. 15, 2009. HFEA Code of Practice that was in effect at the time of donor signature: Edition 8 — R.1. HFEA Code of Practice Edition 7 — R.1 was in effect until Dec. 09, 2007 and Edition 8 — R.1 was in effect: Oct. 01, 2009–Apr. 06, 2010.

Embryo culture and micromanipulation

Embryo culture and laser-assisted dissection of inner cell mass (ICM) were carried out as previously described in details (Ilic et al., 2012; Stephenson et al., 2012). The cellular area containing the ICM was then washed and transferred to plates containing mitotically inactivated human neonatal foreskin fibroblasts (HFF).

Cell culture

ICM plated on mitotically inactivated HFF was cultured as described (Ilic et al., 2012; Stephenson et al., 2012). Trophectoderm cells were removed mechanically from outgrowth (Ilic et al., 2007; Ilic et al., 2010). hESC colonies were expanded and cryopreserved at the third passage.

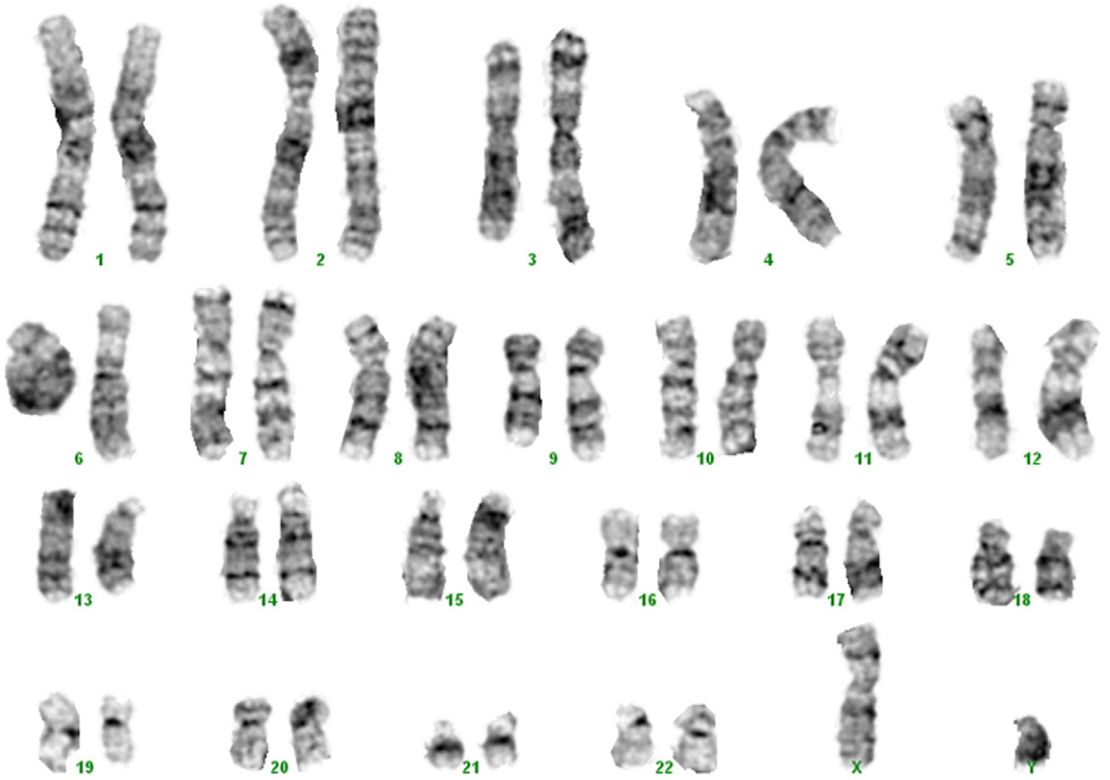

Fig. 2. A modal karyotype (in 19 cells) showed a normal male chromosome complement and banding pattern. In addition, one anomalous cellwas seen (45,XY,−19), believed to be the result of harvesting artifact.

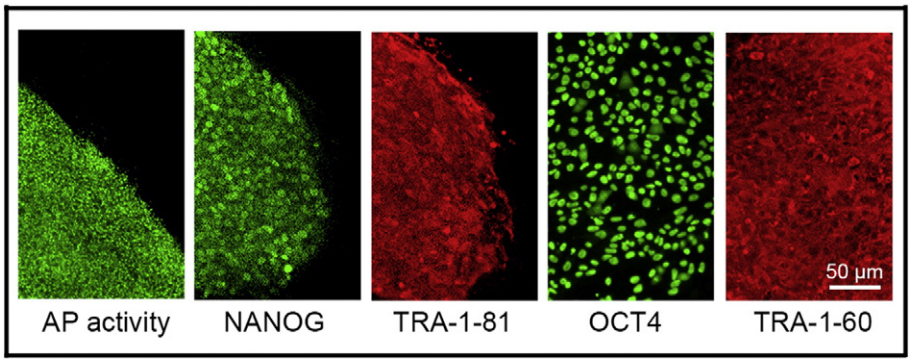

Fig. 3. Expression of pluripotency markers. Pluripotency is confirmed by immunostaining (OCT4, NANOG, TRA-1-60, TRA-1-81) and alkaline phosphatase (AP) activity assay. Scale bar, 50 μm.

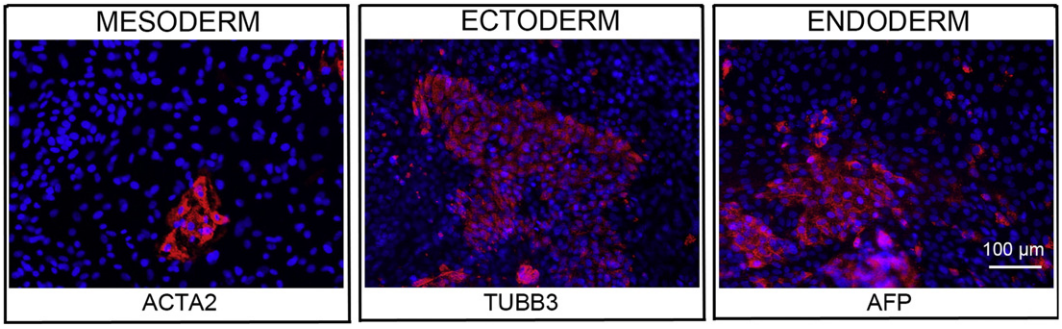

Fig. 4. Differentiation of three germ layers in vitro is confirmed by detection of markers: smooth muscle actin (ACTA2, red) for mesoderm, β-III tubulin (TUBB3, red) for ectoderm and α-fetoprotein (AFP, red) for endoderm. Nuclei are visualized with Hoechst 33342 (blue). Scale bar, 100 μm.

Viability test

Straws with the earliest frozen passage (p. 2–3) are thawed and new colonies are counted three days later. These colonies are then expanded up to passage 8, at which point cells were part frozen and part subjected to standard battery of tests (pluripotency markers, in vitro and in vivo differentiation capability, genetics, sterility, mycoplasma).

Pluripotency markers

Pluripotency was assessed using two different techniques: enzymatic activity assay [alkaline phosphatase (AP) assay] and immunostaining as described (Ilic et al., 2012; Stephenson et al., 2012).

Genotyping

DNA was extracted from hESC cultures using a Chemagen DNA extraction robot according to the manufacturer's instructions. Amplification of polymorphic microsatellite markers was carried out as described (Ilic et al., 2012). Allele sizes were recorded to give a unique fingerprint of each cell line.

Differentiation

Spontaneous differentiation into three germ layers was assessed in vitro and in vivo as described (Petrova et al., 2014; Stephenson et al., 2012).

Array comparative genomic hybridization (aCGH)

aCGH was performed as described in detail (Ilic et al., 2012).

Author disclosure statement

There are no competing financial interests in this study.

Acknowledgments

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